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Usefulness and optimization of mouse models of allergic airway disease

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The usefulness of animal models for studying human asthma has been heavily debated.^{1–12} Although mouse models in particular provide several potential advantages for studying allergic airway disease (Table I),¹³ these advantages disappear if these models differ from the human disease to such an extent that lessons learned from the mouse model are misleading. In this regard investigators who think mouse models are useful have summarized important similarities between experimental mouse allergic airway disease and human asthma (Table II),^{1,5,10,14} whereas investigators who are more concerned that the wrong model will teach the wrong lessons have summarized relevant differences between mouse and human pulmonary biology and between mouse experimental allergic airway disease and human asthma (Table III).^{1,2,4–6,9} Our main purpose here, aside from providing this summary of similarities and differences, is to consider the usefulness of mouse models of allergic airway disease/asthma from a pragmatic and predictive rather than a theoretic standpoint. Specifically, we will (1) consider examples in which the use of mouse models for discovering novel asthma therapeutics has led investigators astray or identified an agent or strategy that proved useful and (2) consider how the use of mouse models for this purpose might be optimized.

Investigators who argue against the usefulness of mouse models of asthma point to the failure of agents identified as potential therapeutics in mouse model studies (platelet-activating factor [PAF], IL-12, and IL-4 and IL-5 antagonists) to be effective therapies for human asthma and the failure of mouse model studies to demonstrate the efficacy of an agent (an IgE antagonist) that later proved useful in human subjects.^{1,8} It is worthwhile to evaluate the data behind these claims. The usefulness of PAF and IL-4 and IL-5 antagonists in mouse models was demonstrated in studies that evaluated their use as prophylactics (ie, they were tested for their ability to inhibit disease development)^{7,9,15,16} and not as therapeutics (ie, agents that suppress established disease). In contrast, human studies evaluated these agents as therapeutics and not as prophylactics.^{7,9,17} The distinction is an important one. For example, IL-4 antagonists effectively inhibit the development of allergic airway disease in the mouse but fail to suppress established mouse allergic airway disease (with the exception of bronchoalveolar lavage eosinophilia).^{9,18} This is probably because IL-4 strongly promotes the differentiation of naive T cells into cells that secrete T_H2 cytokines, including IL-13, but is not required to maintain established T_H2 cytokine

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secretion, whereas IL-13 is more important than IL-4 for inducing and maintaining airway hyperresponsiveness (AHR) and goblet cell hyperplasia.^{9,18} Thus the results of mouse model experiments predicted the failure, rather than the usefulness, of an IL-4 antagonist as a therapeutic for established human asthma.

Additional features of the mouse and human studies are also worth considering. Results of studies with IL-5 antagonists, for example, have differed dramatically when different models have been used. Anti-IL-5 mAb has blocked the development of AHR in some studies in which allergic airway disease was induced in a relatively nonatopic strain (C57BL/6) with a relatively weak allergen (ovalbumin) that was administered systemically with alum adjuvant before it was administered by means of inhalation¹⁹ but not when allergic airway disease was induced in mouse strains in which T_H2 responses are easier to induce (A/J or BALB/c)²⁰ or with stronger allergens (worm or dust mite extracts) that induce allergic airway disease without systemic priming (F. Finkelman, unpublished data). The dissimilar results that are obtained with different mouse strains can be seen as a weakness of the mouse models (eg, How does one know which result applies to human subjects?), but they are also a potential strength. Dissimilar results obtained with different mouse strains reflect mouse genetic diversity that might mirror the influence of polymorphic genes on human asthma. Breeding studies can reveal genes that influence allergic airway disease characteristics and susceptibility in mice; results of these studies might identify genes that have similar effects on human asthma.

A second concern in comparing the results of mouse studies with clinical trials involves limitations of the clinical trials, particularly the care taken to demonstrate that the trials used a sufficient dose of the antagonist to block autocrine and paracrine effects. For example, the dose of soluble IL-4 receptor used as an IL-4 antagonist in human asthma trials was far less than the dose that inhibits most IL-4 effects in mice when the size difference between the 2 species is considered.^{21,22} Furthermore, to the best of our knowledge, none of the human trial data demonstrated effective neutralization of IL-4.

To a lesser extent, the same criticism might apply to PAF antagonist clinical trials, inasmuch as some trials with a potent PAF antagonist did seem to demonstrate efficacy, at least with regard to AHR.²³ Importantly, the rationale for using PAF antagonists as an asthma therapeutic was not entirely based on animal model studies; human studies had shown that inhaled PAF induces bronchoconstriction and AHR, that plasma PAF levels increase during asthma attacks and in response to allergen challenge, that sputum and bronchoalveolar lavage fluid from asthmatic subjects have higher PAF levels than the same fluids in nonasthmatic individuals, that PAF receptor gene expression is increased in the lungs of asthmatic subjects, and that individuals with a genetic defect in the enzyme that breaks down PAF have an increased risk of severe asthma.¹⁵ Thus if animal model prophylaxis experiments misidentified PAF as a good therapeutic target for asthma, so did the initial human studies.

Mouse studies with IL-12, unlike mouse studies with IL-4 or IL-5 antagonists, provided evidence for efficacy, even when used in mice that already had established allergic airway disease.²⁴ An IL-12 trial in human asthmatic subjects showed inhibition of blood and sputum eosinophilia but no objective improvement in pulmonary function when used at a dose considerably less than that used in mice. Unfortunately, even this relatively low dose was associated with unacceptable toxicity (cardiac arrhythmias, abnormal liver function, and a flu-like syndrome, which were not evaluated in the published mouse studies).²⁵ Consequently, the question of whether higher-dose IL-12 might suppress human asthma became moot. Thus none of the experiments with anti-IL-4 mAb, anti-IL-5 mAb, PAF, or

IL-12 that elicited “positive” results in an animal model study were truly comparable with human trials with similar agents that elicited “negative” results.

Mouse model studies have also been faulted for failing to predict that an IgE antagonist could ameliorate human asthma, whereas a nonstimulatory anti-IgE mAb is efficacious in human subjects^{10,26} and has been US Food and Drug Administration–approved as an asthma therapeutic. In this regard some mouse models indeed failed to show any beneficial effect of eliminating IgE or mast cells.⁹ The putative difference between the mouse studies and human trials breaks down, however, when all of the mouse and human data are compared. Mouse models in which strong IL-13 responses are induced by priming mice intraperitoneally with an allergen adsorbed to alum adjuvant and then challenging mice repeatedly with inhalation of the same allergen are characterized by AHR that is both IgE and mast cell independent but IL-13 dependent.⁹ Consistent with this, inhalation of IL-13 (a weak stimulant of IgE production in the mouse) induces AHR in mice that lack B cells, T cells, and antibody.^{18,27} Both IgE and mast cells, however, contribute to the development of AHR in mouse models that forgo systemic priming with allergen and, consequently, are likely to induce less IL-13 production.⁹ Taken together, these mouse model studies indicate that IgE and mast cells can contribute to, but are not necessary for, allergic airway disease. Human experience has not been very different: anti-IgE mAb can improve quality of life and decrease symptoms, severe exacerbations, and steroid use (parameters difficult to measure in animal models) in most asthmatic subjects but does not significantly improve the results of objective pulmonary function testing.^{26,28,29}

Although the apparent differences between the usefulness of all of these agents in human asthma versus the mouse models can thus be explained, these explanations by themselves do not justify the usefulness of mouse models. A recent human study, however, might provide such justification. Agents that block both IL-4 and IL-13 by inhibiting the function of IL-4 receptor α , the signaling polypeptide of the IL-4 and IL-13 receptors, suppress allergic airway disease in both prophylactic and therapeutic mouse models, provided that a sufficiently potent agent is used in sufficient dose.^{30,31} Recent studies of human asthmatic subjects with a mutant form of IL-4 that is an IL-4 receptor α antagonist demonstrate consistent results, including improvement in objective pulmonary function testing.³² Although these human results require confirmation by other investigators and other IL-4 receptor α antagonists, they hold promise that a novel agent, developed as a result of mouse model studies, will improve life for persons with asthma. Furthermore, although this might be the first example in which mouse model studies have predicted the success of an agent for treatment of human asthma, there are other examples of agents that suppress both human asthma and the mouse models, including corticosteroids, leukotriene/leukotriene receptor inhibition, and phosphodiesterase 4 antagonists.¹

In addition, even when mouse studies fail to promise a likely therapeutic benefit for patients with asthma, mechanistic information gained from the mouse studies might improve overall understanding of the processes involved in asthma pathogenesis and might even be useful for treating other human diseases. For example, the strong antieosinophil effect of anti-IL-5 mAb in mice suggested that it might be useful for treating patients with hypereosinophilic syndrome, eosinophilic dermatitis, eosinophilic nasal sinus disease, and eosinophilic esophagitis; the results of initial clinical studies are consistent with this suggestion.^{33–36}

Finally, we believe that the similarities and differences between human asthma and the mouse models and the results of the above-mentioned mouse model studies suggest ways to optimize mouse (and other animal) models for predicting whether a particular agent or intervention will be efficacious for human asthmatic subjects (Table IV). Although we hope that positive results with mouse models that adhere to these suggestions will increase the

chance that the agents studied will be useful in human subjects and thus make the selection of agents for human trials more effective and efficient, all proponents of animal studies recognize that prospective, randomized, blind, sufficiently powered, and properly designed human studies are required to establish such usefulness.

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TABLE I

Potential advantages of mouse models

1	Ethical considerations drastically limit human experimentation with agents of unknown efficacy and <i>in vivo</i> toxicity.
2	The great expense of human trials relative to animal studies makes it advantageous to screen potential agents with studies in other species before testing them in human subjects.
3	Mice are plentiful, relatively inexpensive, and easy to breed and house.
4	There are fewer regulatory concerns for <i>in vivo</i> studies of mice than for <i>in vivo</i> studies with most other vertebrate species.
5	There is availability of inbred mice of several different strains that have defined immunologic and airway physiologic properties, including differences in susceptibility to allergic airway disease and features of allergic airway disease.
6	Specific reagents for experimental studies (eg, mAbs and polyclonal antibodies, soluble receptors, PCR primers, and inhibitory RNAs) are more readily available for mice than for other experimental species.
7	Transgenic overexpressor and gene-targeted mice, including cell type-specific and inducible strains, are available or producible.
8	The complete DNA sequence for the mouse genome is known, ¹³ and resources for determining the expression of all mouse genes are available.

TABLE II**Similarities of mouse models to human asthma**

1	Allergen inhalation induces responses that are broadly similar to those found in human asthma:
a.	T_H2 cytokine production (IL-4, IL-13, and IL-5)
b.	Goblet cell hyperplasia
c.	Bronchoalveolar lavage and pulmonary neutrophil response, followed by eosinophil response
d.	Mast cell degranulation
e.	IgE production
f.	AHR to cholinergic stimuli
g.	Rapid and delayed development of increased airway resistance. ⁵
2	Both human asthma and mouse models show memory responses. ¹⁰
3	Some mouse models have chronic “remodeling” with smooth muscle hyperplasia and subepithelial fibrosis. ¹⁴
4	Both human asthma and at least some mouse models are suppressed to some degree by leukotriene antagonists and leukotriene receptor antagonists, mast cell depletion, IgE antagonists, phosphodiesterase 4 antagonists, and corticosteroids. ¹
5	Intraspecies genetic variability influences susceptibility to allergic airway disease in both mice and human subjects. ⁵

TABLE III**Differences between mouse models and human asthma**

1	Asthma does not spontaneously develop in mice. ⁹
2	Mice have 6–8 generations of branching airways with no gas-exchanging respiratory bronchioles, whereas human subjects have 20–23 (or 27–29) generations of airway branching with gas-exchanging respiratory bronchioles. ¹
3	Mouse airways are narrower in absolute terms but wider relative to body size than human airways. ²
4	Unlike human airways, most mouse airways beyond first-generation bronchi lack smooth muscle bundles. ¹
5	Mouse airways, except the trachea, lack submucosal mucus glands, whereas these are abundant in human large- and medium-sized airways. ¹
6	Mice have a more compliant chest wall and lower functional residual capacity. ³
7	The mouse lung is more fully developed than the human lung at birth. ¹
8	Mouse eosinophils, unlike human eosinophils, show little degranulation. ⁵
9	Eosinophils are distributed differently in mouse models vs human asthma. ⁶
10	Vascular and perivascular inflammation are prominent in mouse models but are missing in human asthma. ¹
11	Clara cells account for a greater percentage of airway epithelium in mice than in human subjects. ⁶
12	Testing for AHR is very different in mice vs human subjects. ¹
13	Mice are housed under different (and generally cleaner) conditions than human subjects. ²
14	Mice and human subjects respond differently to many mediators (eg, neurokinins, histamine, and leukotriene C ₄ can cause bronchoconstriction in human subjects but not in mice). ⁴
15	Not all effects of T _H 2 cytokines are similar in mice and human subjects. ¹

TABLE IV**Suggestions for optimizing the applicability of mouse model studies to human asthma**

1	Consider known differences between mouse and human immunology, anatomy, and physiology when deciding whether studies should be performed with a mouse model.
2	Evaluate whether an agent or intervention will suppress already established allergic airway disease (therapeutic usefulness). This limits the use of gene knockout mice to mice in which the gene can be inducibly deleted but allows the use of several types of antagonists (antibodies, soluble receptors, inhibitory RNAs, and mediator antagonists and enzyme antagonists).
3	When using an antagonist, determine whether a sufficient dose was used to optimally inhibit the target (equally true for human studies).
4	Use a potent allergen that can stimulate allergic airway disease when administered by means of inhalation without prior systemic priming.
5	Use a mouse strain that is relatively susceptible to atopic disease (ie, relatively likely to generate a strong T _H 2 cytokine response to airway immunization).
6	Use both invasive and noninvasive methods to establish AHR (see recent <i>Journal of Allergy and Clinical Immunology</i> editorial ³⁷).
7	Confirm results with at least 1 additional model, if possible.